The Relationshipe Between Verapamil and Stress Ulcer in Rats

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Özet: VERAPAMİL VE STRES ÜLSER İLİŞKİSİ
Mide stres ülserlerinin oluşmasında rol alan çeşitli etiyolajik faktörler, kalsiyumun hücre içine akşını stimüle etmektedir. Stres ülserlerinin patogenezinde hiperasiditenin rolü olduğu bilinmektedir. Kalsiyum kanal blokerlerinin stimüle edilmiş asit sekresyonunu inhibe ettiği kliniğimizdeki çalışmalarla gösterilmiştir. Bu nedenle verapamil'in stres ülserlerinin önlenmesindeki etkileri plasebo kontrollü bu çalışmada ranitidin ile karşılaştırmalı olarak incelenmiştir. Araştırmaya alınan 30 adet erkek sıçan (150-200gr) 24 saat aç bırakıldı, ancak su içmelerine izin verildi. Sıçanlar, herbiri 10 sıçan içeren 3 gruba ayrıldı. Plasebo olan 1. gruba 1 ml serum fizyolojik (SF), 2. gruba ranitidin 3mg/kg, 3. gruba ise verapamil 20mg/kg aynı hacımdeki SF ile intraperitoneal (ip) olarak enjekte edildi. İlaçların verilmesinden 1 saat sonra tüm sıçanlara 4 saat süre ile hareketsizlik stresi uygulandı. Deney sonunda hayvanlar öldürüldü, laparotomi yapılarak çıkarılan mideleri büyük kurvatur boyunca açıldı. Lümendeki kan ve mukozadaki peteşi sayısı değerlendirildi. Santimetrekareye(cm2) düşen peteşi sayısı: 0-2=1, 3-4=2, 4>=3 4>+kanama= 4 olarak indekslendi. Peteşi indeksi plasebo grubunda 3.75±0.67 iken verapamil grubunda 3±0.63 ile %22.2 azalma gözlendi. Gruplar arasındaki fark istatistiki bakımdan anlamsızdı. Ranitidin ise plaseboya göre mide lezyonlarını %73.4 önledi ($p<0.01$) ve peteşi skoru 1±0.05 idi. Bulgular stres ülserlerinin önlenmesinde verapamil'in anlamlı oranda etkili olmadığını, ranitidine'in ise potent bir şekilde koruduğunu göstermektedir. Bu sonuçların verapamil dozuna bağlı olarak yapılacak yeni çalışmalarla desteklenmesini ümit etmekteyiz.

Anahtar Kelimeler: Stres ülser, verapamil, ranitidine, tavşan.

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Summary: The relationshipe between verapamil and stress ulcer on restraint-stress ulcer formation was studied in rats. Thirty male albino rats were used for this experiments. Animals were divided into three groups. Group 1 an intraperitoneal (ip) injection of 0.9 % saline 1 ml, group 2 received an ip injection of verapamil 20 mg/kg group 3 received an ip injection of ranitidine 3mg/kg. One hour later after treatment, all animals were restrainted for four hours. Gastric mucosa was inspeted for lesions, and the ulcer index evaluated. Untreated animal showed multiple lesions of various localisation and size in stomach. In this group the mean lesion index was 3.75±0.67. Intraperitoneal administration of verapamil reversed 22.2 percent the effect of restraint-stress induced gastric lesions. In this group lesion index was 3.00±0.63. Administration of ranitidine abolished 73.4 percent gastric lesions. In this group the mean ulcer index was 1.00±0.05($p<0.01$). In conclusion verapamil 20mg showed no significant difference from placebo in preventing mucosal damage, was induced by stress ulcer formation. Ranitidine significantly reduced gastric lesion. There are many possible reasons why such a results are suprising in view of verapamil as a anti-ulcer drug. Future studies to further evaluate anti-ulcer effect are recommended. Although there are many problems comparing experimental results in animals with clinical investigations the conclusion may be allowed that verapamil could open new possibilities in the treatment of ulceration.

Key Word: Stress ulcer, verapamil, ranitidine,rat.

Histamin is a critical component of gastric function as well as in disease states such as gastroudodenal ulcer. Histamine exerts its ef-

fects in the gut through specific receptors(histamine H_2 receptors) and second messenger systems which result in the accumulation of intracellular calcium. Since calcium appears to be an important component of histamine's action, several researchers have begun investigating calcium channel antagonists with respect to their effects in both normal and stress-challenged gut function[1]. It was reported that verapamil decreased restraint-induced ulcers whereas the same compound exacerbated ethanol-induced gastric mucosal damage[2]. Subsequently some investigators reported that verapamil inhibited gastric acid accumulation in pylorus-ligated rats, but only at low doses[3]. A dose of verapamil which did not influence gastric acid secretion retained its anti-ulcer effect. However, using the rat isolated, perfused stomach, was found that verapamil did not affect gastric secretion[4]. The anti-ulcer effect of verapamil was confirmed and also noted that this compound decreased plasma gastrin levels in stressed rats[5]. Recently, we replicated, preventive effect of aspirin-induced gastric damage amelierating of verapamil. We now report that possible protective action of verapamil and ranitidine against gastric mucosal injury induced by restraint stress and to compare this effect to the protection provided with each other.

MATERIALS and METHODS

Male albino rats 150-200 g were used for the experiments. Animals were fasted for 24h, but allowed access to water ad libitum.

The animals were divided into three groups. Group 1(n:10) an intraperitoneal (ip) injection of 0.9% saline 1ml, group 2(n:10) received an ip injection of verapamil 20mg/kg, group 3 (n:10) received an ip injection of ranitidine 3mg/kg.

One our later after treatment, all animals were restrained for 4 hours by a standard procedure according to Brodie and Hanson[6].

Table I: Gastric lesion score after 4 hours restraint-stress.

Groups	Score	Preventive effect (%)
Placebo	3.75±0.67	00.00
Verapamil	3.00±0.63**	22.20
Ranitidine	1.00±0.05*	73.40

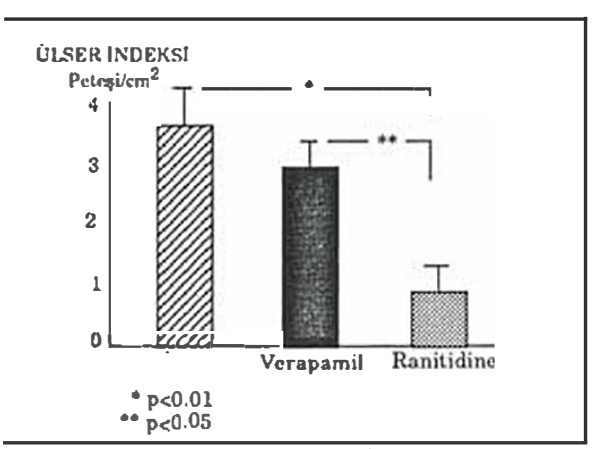
*p<0.01 compare to placebo
**p<0.05 compare to ranitidine

After each experiment, animals were sacrificed by air embolism. Stomachs were quickly removed and opened along the greater curvature. Gastric mucosa was inspected for lesions and the ulcer index evaluated. With the aid of dissecting microscope(x100), we calculated the average length of each lesion in mm and used this figure as the ulcer index[6].

Student's t-test was used for statistical analysis.

RESULTS

After 4 hours of immobilisation all untreated animals showed multiple lesions of various localisation and size in stomach. In this group the mean lesion index was 3.75±0.67. Intraperitonial administration of verapamil reversed the effect of restraint stress induced gastric lesions. In this group the mean ulcer index was 3.00±0.63, but the preventive effect of verapamil, on stress ulcer production, was not significant difference from control group(Table I, Graphic 1). Ranitidine abolished 73.4 percent



Graphic 1: Gastric lesion score after 4 hour restraint-stress

gastric lesuons. In this group the mean ulcer index was 1.00±0.05($p<0.01$).

DISCUSSION

This study showed a statistically significant advantage of ranitidine to verapamil in reducing gastric mucosal lesions. Otherwise, verapamil showed no significant difference from the placebo in preventing mucosal damage from restraint stress-induced gastric damage. There are many possible reasons why such a result may have occured, but the results are suprising in view of the strong positive experimental evidence of verapamil. Future studies to further evaluate for preventive effect of verapamil are recommended.

However, in this study, verapamil decreased 22.2 percent gastric damage induced by restraint-stress. The findings with verapamil are consistent and extend the anti-stress ulcer properties of verapamil over a wider dose range[1-3,7].

Histamine is a critical component of gastric function as well as in disease states such as gastroduodenal ulcer. Histamine exerts its effects in the gut through specific receptors(Histamine H_2 receptors) and second messenger systems which result in the accumulation of intracellular calcium. Since calcium appears to be an important component of histamine's action, several researchers have begun investigating calcium channel antagonists with respect to their effect of both normal and stress challenged gut function[1].

Calcium plays an important role in the regulation of gastric acid secretion[8-13]; however the exact mechanism by which calcium influences parietal cell is unknown. It remains controversial whether any of the three classes of calcium channel antagonists can influence gastric acid secretion. Previous work by our clinics and others showed that parenteral verapamil inhibited pentagastrin or histamine-stimulated gastric acid acid secretion in human and also reduced gastric acid secretion and stress-and ethanol-induced gastric ulcer in rats[1,7,14-18]. In contrast, others demonstrated that verapamil did not alter basal or near-maximal pentagastrin-stimulated gastric acid secretion in parietal cell by verapamil may not involve calcium channel antagonism[19].

Rather, these drugs inhibit directly the H^+,K^+-ATPase pump under acidic conditions by interfering with the high affinity K^+-site of the H^+,K^+-ATPase system, which is accessible from the luminal side of the stomach[19]. It appears that the reactive species of this weak base is the protonated charged from. In the intact cell, in protonated form to a luminal rather than a cytosolic site could prevent acid secretion[19]. The inhibitory action of verapamil on histamine-stimulated parietal cell was reserved after buffering the acid spaces by imidazole[19]. Two possibilities were considered to explain such findings. First, verapamil is a weak base, its accumulation could be reduced as the pH of the canalicular space was elevated by imidazole. Second, verapamil's activity is reduced in its non-protonated or neutral form.

Verapamil inhibited H^+K^+ -ATPase and PNPase activities in competition with K^+[22]. The inhibition of these enzymes were pH sensitive[20].

Inhibition of parietal cell H^+,K^+ -ATPase represents a potential beneficial property in development of future antiulcer drugs, as indicated by the substituted benzimidazoles[21,22] and by a new group of sulfoxide agents, both of which have also proven to be potent inhibitors of gastric H^+,K^+ -ATPase[23].

In conclusion, although there are many problems comparing experimental results in animals with clinical investigations, the result may be allowed that verapamil could open new possibilities in the treatment of restraint stress-induced gastric lesions.

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